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Crohn's Disease Management: From Classical Approaches to Recent Advances

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ABSTRACT

Crohn's disease (CD) is a lifelong debilitating condition. Different theories have been proposed regarding the aetiology of the disease like genetic factors, microbial factors and in recent years there is increased interest in the role of *Mycobacterium* in this disease. The high global incidence of this disease has been reported. The disease was rare till 1980s in India, however, past few decades has seen an increased prevalence. Presently available treatment offers symptomatic relief. Surgery is suggested in advance cases. However, frequent relapses of the disease have been observed. Therefore, therapeutic development for CD is a hot area of research and many new avenues are being explored. Here we review the classical and modern advances in managing this disease.

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INTRODUCTION

Crohn's disease (CD) has been a major form of chronic inflammatory bowel disease that may affect the gastrointestinal tract most commonly the ileum, colon and perineum. The major factor associated with the disease is segmental and transmural inflammation of the intestinal wall associated with tissue damage and inflammatory lesions (Barbara *et al.,* 2012). There are five types of CD based on the part of the GI tract that is affected, namely, Ileocolitis (affects ileum & colon), Ileitis (affects only ileum), Gastroduodenal Crohn's disease (affects stomach & duodenum), Jejunoileitis (patchy areas of inflammation in jejunum), Granulomatous colitis (affects colon only) (Barbara *et al.,* 2002). Symptoms associated with CD depend on the type, however generally symptoms include loss of appetite, weight loss, weakness and anaemia. The disorder may affect several age group, but the beginning is mainly common among teenagers as well as young adults. During diagnosis, differentiating CD from other inflammatory bowel diseases is essential as it can complicate the required clinical course. Diagnostic tests include a medical history followed by a physical examination, a blood test, a stool test and colonoscopy among others. Once the patient has been diagnosed with CD, the Crohn's Disease Activity Index (CDAI) is calculated to quantify the symptoms of the patients with CD and based on the CDAI value medications are altered and the state of the progress of the disease is noted.

It was observed that industrial countries have a higher prevalence of Crohn's Disease than in developing countries. However, the prevalence rate of the disease in countries like China and India are rapidly increasing suggesting lifestyle as one of the main factors contributing to the onset of the disease.CD is caused by a combination of factors such as genetics, the immune system, smoking, previous infection and various types of environmental stresses. The condition is unpredictable with ma-

jor variations among patients and its higher prevalence being reported in recent years. The disease is highly regarded as an emerging global problem.

Treatment continues to remain a challenge in the CD. Most of the treatment options aim to reduce inflammation, eliminate nutritional deficiencies and relieve the symptoms of pain, diarrhoea & bleeding which accompany the disease. No single treatment available can be considered universal and currently large number of clinical trials are being carried out worldwide. Many time's patients do not respond to standard treatments and hence new alternatives need to be sought after to relieve the symptoms. From an autoimmune disorder to bacterial infection, the CD is classified under various categories due to its uncertain aetiology. The review focus of the various available treatments for CD as well as possible treatments that could be a solution to this rather complex disease.

Therapies for CD

The use of medications, change in diet and nutrition and surgery in extreme cases are some of the methods by which CD is currently being treated. Medical treatments for CD have two main goals, (i) attaining remission and (ii) maintaining remission. Several classes of drugs have been approved over the years to attain these goals in CD treatment. Brief descriptions of these drugs are mentioned below.

Aminosalicylates

Aminosalicylates are anti-inflammatory drugs that are often used as the first line of treatment for inflammatory bowel diseases like CD and ulcerative colitis. Being used for over 70 years, aminosalicylates contain 5-aminosalicylic acid (5-ASA) that reduces inflammation in the lining of the intestine. Some forms may also reduce joint inflammation. 5-ASA primarily exhibits a topical effect on the colonic epithelium, often very well tolerated in patients and have not been associated with an increased risk of infection or cancer. The first 5-ASA commonly used in IBD was sulfasalazine (Azulfidine®) but is known to have a high level of intolerance due to the presence of sulfa component. However, sulfasalazine is the standard recommended treatment for colonic CD as per clinical practice guidelines in countries such as Germany (Klang *et al.,* 2015). Mesalamine, a sulfa-free derivative of sulfasalazine was developed and is considered to exercise its effect through inhibition of cyclooxygenase and lipoxygenase (Hahn *et al.,* 2015).

In 2015, data collected from the Swiss Inflammatory Bowel Disease Cohort analysed the role of 5-ASA in the treatment of CD from 2006 and revealed that 46% (378/825) of treatment episodes were reported as successful. However, 12% (98/825) of the treatment had been stopped due to the side-effects (Schoepfer *et al.,* 2015). Yang and co-workers performed a meta-analysis on the efficacy of 5-ASA for the prevention of post-operative repetition in Crohn's disease. The findings revealed that 5-ASA was superior in comparison to placebo for prevention of clinical recurrence, without increasing the rate of side effect.5-ASA Good tolerance rate among patients and low frequency of side effects, make it the most favoured method of treatment commonly used in the mild and colonic CD. However, the major drawback is its lack of effect in small bowel and upper GI regions, severe and fistulising CD and its inability to cause remission Though the level of intolerance in patients is low, recent reported side effects like hepatotoxicity, exacerbation of colitis symptoms and myocarditis followed by the immediate improvement on discontinuation of mesalamine highlights the need of constant monitoring of patients undergoing the treatment drug (Nair *et al.,* 2015; Hahn *et al.,* 2015;Tuna *et al.,* 2015). Recent researches have highlighted the need to be used in conjunction with other therapies to control inflammation and prevent complications in CD adequately.

Corticosteroids

Along with aminosalicylates, the first line of defence when a patient is diagnosed with CD is the administration of steroids to reduce inflammation. Corticosteroids, introduced as a therapy in the 1950's have been used for decades as anti-inflammatory drugs and continuously used to prevent a flare-up of the disease. These drugs also reduce the activity of the immune system, which is believed to be heightened during the manifestation of the disease. The heightened immune response causes the infiltration and activation of several inflammatory and immune cells, which release many inflammatory mediators that interact and activate structural cells at the site of inflammation. Corticosteroids inhibit the synthesis of these proteins through suppression of the genes that encode them (Barnes *et al.,* 2014). Though corticosteroids are effective for induction of remission of CD, patients are known to relapse when steroids are withdrawn or become steroid dependent with significant side effects like kidney disorders, nausea, vomiting and skin rashes etc. (Alfadhli *et al.,* 2005).

Methotrexate is a cytotoxic anti-inflammatory drug that exerts its benefits through the inhibition of dihydrofolate reductase. It is used to treat certain cancer type's severe psoriasis and rheumatoid arthritis. Over the years, many controlled trials were carried out to evaluate the efficacy of the drug. Earlier works involve the study by Kozarek and co-workers where the anti-inflammatory effect of the drug was evaluated (Kozarekat al.,

1989). 11 of 14 patients with CD showed a good response towards the drug. A study in 2000 was conducted to evaluate the long-term efficacy of the drug and it was revealed that 41 of the 49 patient had attained complete clinical remission with long-term benefits of maintenance treatment in patients with chronically active Crohn's Disease with minimal side-effects (Lémann *et al.,* 2000). Scherkenbach and Stumpf in 2015 evaluated the ability of methotrexate to treat children with CD based on the literature available and concluded that methotrexate is useful in the treatment of paediatric CD but must be administered only when other therapies fail and that the remission rate of CD is comparable to that of thiopurine therapy (Scherkenbach and Stumpf, 2015). Methotrexate was also found to be an efficient alternative in patients where azathioprine treatments have failed (thiopurine resistant patients) (Hojsak *et al.,* 2015). In the study, of the 32 patients, 22 remained in the stable clinical remission after 12 months while 14 did not relapse in 5 years. Recently, an experiment from randomised trial reveals that administration of intramuscular methotrexate (25mg/week) provides a benefit for induction of remission and complete withdrawal from steroids in patients with refractory CD. This data supports the consensus of the positive benefits of methotrexate. The most recent data suggests that methotrexate is effective based on of initial response in CD cases which have failed, or intolerant to thiopurines.

Nonetheless, the study noted that in the long run the efficacy was not sustained (Suares *et al.,* 2012). Multicenter studies have reported that subcutaneous administration of methotrexate was superior to oral administration especially in the case of the paediatric CD (Turner *et al.,* 2015). The drug has found favour in many studies as an alternative to standard therapy. A study in 2015 also found methotrexate to be an effective therapeutic alternative in the thiopurine-resistant patients ultimately leading to complete mucosal healing.

Though the results are promising, the major drawback of the drugs is the side effects it poses on the patients. Methotrexate is not recommended during pregnancy as the drug can cross the placenta and posses a serious risk to the fetus. It has been labelled as category X by the FDA due to its tetragonal nature (Curry and Moss., 2015). Patients who receive the drugs are instructed to delay conception as many deformities like large fontanels, craniosynostosis, abnormal head shape, hypertelorism, and skeletal deformities have been reported. Methotrexate is mostly recommended as an alternative to standard drugs owing to the side effects. The immunosuppressive, anti-inflammatory drug inhibits the actions of folic acid and has been used for the treatment of several autoimmune disorders like rheumatoid arthritis and lupus as well as for the treatment of cancer.

Anti-TNF therapy

Monocytes, macrophages and T cells mainly produce human TNF-α protein involved in immune regulation. TNF-α is released due to various stimuli like endotoxin, superantigens, osmotic stress, radiation etc. TNF-α is recognized for its involvement in recruiting circulating inflammatory cells to local tissue sites of inflammation, for the induction of oedema and its crucial role in granuloma formation. The inflammatory reaction in Crohn's disease is presumably initiated and propagated by local release of cytokines. During exacerbation of inflammatory bowel disease production of interleukins and TNFcx is increased.

Derkx and co-workers performed the first report of TNF treatment for CD in 1993 (Derkx *et al.,* 1993). In this study, the case of a girl with CD who was unresponsive to medical treatment was reported wherein due to the persistence of severe illness. She was administered with anti-TNFα (chimeric monoclonal, cA2). The treatment showed marked improvement immediately after the first dose of anti-TNF, with the improvement of clinical symptoms and complete colonoscopic remission attained for 3 months after which the patient relapsed. Since then anti TNF drugs like Adalimumab, Infliximab have been developed and are getting much attention. Adalimumab and infliximab are both monoclonal antibodies that have a high affinity for TNFα. They have been recommended by the UK's National Institute for Health and Care Excellence as treatment options for severe active Crohn's disease that has not responded to conventional treatment (including immunosuppressive and corticosteroid treatments). Though convincing they carry the risk of opportunistic infection as highlighted in recent research that showed that anti-TNF therapy doubles the risk of opportunistic infections in inflammatory bowel disease patients (Ford *et al.,* 2013).

Infliximab (Brand Name: Remicade)

It is a chimeric anti-TNF monoclonal antibody with anti-inflammatory properties. Over the years, various controlled trials have demonstrated a high level of efficacy of infliximab in both active and fistulating CD. Studies have also revealed that for CD patients not responding to conventional treatments long-term treatment with infliximab show high efficacy and tolerability in managing symptoms (Rutgeerts *et al.,* 1999). The drug has undergone numerous clinical trials for the past 20 years.

Currently, the drug is used for the treatment of refractory luminal CD, steroid-dependent CD, and refractory fistulizing CD.

The limiting factor of infliximab is that some patients do not respond to infliximab while others lose their response over time. This was thought to be due to the drugs tend to induce the development of anti-infliximab (anti-IFX) monoclonal antibodies (mAbs). These antibodies were thought to act against the drug and reduce the chances of patients attaining clinical remission by decreasing the drug survival and as a result requiring a higher drug dose. In a study in 2005, at the John Hopkins Arthritis Centre, the effect of infliximab on CD patients was evaluated and concluded that patients who develop anti-infliximab antibodies are at higher risk for showing a lower duration of response to treatment with infliximab. A similar study by Steenholdt and co-workers evaluated 106 patients over a 10-year course who were treated with infliximab. They observed that patients with high infliximab trough levels and low anti-IFX antibodies were able to maintain a good response to the infliximab maintenance treatment. However, patients with low infliximab trough levels and high anti-IFX antibodies lost their response to infliximab treatment (Steenholdt *et al.,* 2011). Similar multiple studies have linked the loss of response to infliximab to the rise in anti-IFX antibodies (Baert *et al.,* 2003; Farrell *et al.,* 2003; Ternant *et al.,* 2008; Candon *et al.,* 2006). In 2014, Levesque performed a study to determine the link between serum infliximab concentration and efficacy of the drug in CD patients by setting a cut off value of trough infliximab concentration. The study confirmed the relationship between the two and observed that infliximab trough concentrations below 3 μg/mL have a greater chance of developing symptoms for CD (Levesque *et al.,* 2014). However, in a systematic review by Cassinotti noted that though infliximab trough concentration could be used in determining the immunogenetics of the drug, the data available among different research groups have enormous variations in the methods of determining the antibody levels that it no conclusion can be made about the effects of anti-IFX antibodies on the efficacy of infliximab (Cassinotti *et al.,* 2009).

Adalimumab

By early 2000, it came to be known that full human monoclonal antibodies are less immunogenic than chimeric monoclonal antibodies. This bought the attention to alternatives to infliximab (chimeric monoclonal antibodies) as CD patients have been reported to become less responsive to the drug over the course of treatment. Adalimumab is a full human immunoglobulin G1 (IgG1) monoclonal antibody that targets membrane and soluble TNF explicitly and binds with high specificity but not to lymphotoxin. Adalimumab has been shown to be effective for disease such as rheumatoid arthritis, psoriasis, and ankylosing spondylitis among others. Adalimumab (HUMIRA®, Abbott Laboratories, and Abbott Park, Illinois, USA) has shown to be beneficial to patients who lose the response to infliximab. In a randomized 4 week clinical trial in 2006 (CLASSIC I), the efficacy of adalimumab was evaluated for the CD patients. The results revealed that the drugs were well tolerated and the patients were able to attain higher remission in comparison to placebo. In the following year, the CLASSIC II trial evaluated the long-term efficacy and safety of adalimumab maintenance therapy in CD cases. The study reported that 93% of the patients attained remission for up to 56 weeks. Following the initial studies, numerous clinical trials have shown the successful attainment of remission in CD patients.

Like Infliximab, studies have shown that Adalimumab can induce and maintain mucosal healing in CD patients as well. This was highlighted by Rutgeerts and co-workers in a clinical trial which observed mucosal ulceration through ileocolonoscopy during treatment by adalimumab for 52 weeks and compared it placebo-treated patients, whom all went through a 4-week induction therapy with adalimumab. Initially all patients received induction therapy with adalimumab at week 52, 0 and 24% healing was observed for placebo and adalimumab administered patients highlighting that patients who continue to receive adalimumab after induction therapy with adalimumab have a higher chance of attaining remission than those who do not continue the drug administration (Rutgeerts *et al.,* 2012**).** A 2007 study by Sandborne highlighted the lack of antibody production against adalimumab in all 159 CD patients, a factor greatly considered as the reason for the loss of response against drugs like infliximab (Sandborne *et al.,* 2014; William *et al.,* 2007).

Being an anti-TNF drug, the side effects observed are those that due to the lowering of the immune system and becoming more prone to other infections. Few cases of drug-induced psoriasis and latent tuberculosis have also been reported. The most known side effects are injection site reactions a headache and nausea (Cassinotti *et al.,* 2008). The drug is well tolerated among patients while the safety and tolerability of adamulimab are similar to infliximab. The main advantage of adalimumab has the subcutaneous mode of drug delivery. This non-invasive method is easy to use and can be done by the patient itself.

Natalizumab

Natalizumab is a humanized monoclonal antibody, that is directed against the α4 subunit of integrin heterodimers and functions by inhibiting the binding of both a4b7 integrins, located primarily in the vasculature of the gut, and a4b1 integrins, located primarily in the vasculature of the brain. The drug was used for the treatment of multiple sclerosis and Crohn's disease. A double-blind, placebo-controlled trial where 248 patients with moderate-to-severe Crohn's disease were treated with natalizumab was carried out in 2003 by Ghosh and co-workers (Ghosh *et al.,* 2003). The results revealed that though statistical significance could not be attained between the placebo and test groups, at week 4 and 8 at of the trial the rate of remission was comparatively higher than the placebo groups. Though the results were promising, in a controlled trial in 2005 that evaluated the use of natalizumab as induction and maintenance therapy in patients with active CD, one patient died from progressive multifocal leukoencephalopathy (PML), associated with a human polyomavirus (JC virus) (Sandborn *et al.,* 2005). The death of the patient leads to Gert Van Assche and co-workers to analyze the frozen serum samples and the results showed that JC virus DNA was present in the patients serum three months after natalizumab was administered and two months before the appearance of symptomatic PML (Gert Van Assche *et al.,* 2005). The report also noted staining of the brain lesion for polyomavirus and suggested the possibility that the anti–α_4-integrin therapy could result in JC virus-induced PML. The drug was pulled from the market and re-introduced in 2008 after no further cases of PML were observed in all the clinical trials. The single patient who dies of PML was later understood to be have been under immunosuppressive drugs at that time and hence through the drug is approved, strict laws about the usage of nazaminab are in place and the drug can be used only if the patients are non-responsive to available treatments. Sakuraba in 2013, assessed the efficacy of natalizumab for the management of CD outside the settings of a clinical trial in a tertiary hospital in the USA (Sakuraba *et al.,* 2013) of the 49 patients receiving,the drug no case of PML was reported however, 5 patients experienced server-side effects. The drug was concluded to be a safe alternative to a patient who does not respond to TNF treatment. Similar results were observed by Pascal and co-workers, where the drug was concluded to be safe and with high efficacy and clinical improvement for more than one-third of more than a year in one-third of CD cases who did not respond to conventional therapy (Juillerat *et al.,* 2013).

Certolizumab pegol

Approved by The FDA in 2008 Certolizumab pegol (CDP870, tradenameCimzia) is a therapeutic monoclonal antibody to tumour necrosis factor alpha(TNF-α), for the treatment of Crohn's disease. Various clinical trials have demonstrated its positive effect on the treatment of CD. 3 phase III (PRECiSE I PRECiSE II PRECiSE III) multicentre, placebo-controlled, double-blind, randomised trials have been conducted and demonstrated that Certolizumab pegol is sufficient for induction and maintenance of clinical response in patients with moderate to severely active CD (Sandborn *et al.,* 2014).In order to evaluate the efficacy and safety of Certolizumab pegol outside the clinical setting, Moon and co-workers evaluated patients who received CZP between 2008 and 2013 through retrospective medical record-based review of steroid-free complete response (SCR), loss of response and safety (Moon *et al.,* 2015). Certolizumab pegol was found to be both effective and well tolerated for the treatment of Crohn's disease. Miguel and co-workers evaluated the effect of smoking during the treatment of Certolizumab pegol for CD and confirmed that smoking had a negative impact on patients with CD (Miguel *et al.,* 2015).

A meta-analysis in 2013 compared the effectiveness and safety of, adalimumab and certolizumab with either a placebo or each of them in the treatment of CD than and concluded that all the drugs were effective as both induction and maintenance therapy in moderate to severe Crohn's disease in adults, with an acceptable safety profile (Kawalec*et al.,* 2013).

Ustekinumab, another monoclonal antibody works against the p40 subunit of interleukin-12 and interleukin-23 that targets both the T-helper 1 and T-helper 17 pathways involved in the pathogenesis of CD, has also been explored. Ustekinumab has already been approved for the treatment of plaque psoriasis and psoriatic arthritis but not for a CD. A retrospective observational study in 2016 concluded that almost two-thirds of patients with CD refractory to at least 1 anti-TNF agent received clinical benefit from ustekinumab therapy (Wils et al. 2016). The patients did not require steroids for up to 12 months afterwards. The study recommended the use of ustekinumab in these refractory CD patients. In regards to anti-TNF resistant CD, a Mexican study in 2016 concluded that patients with moderate to severe anti-TNF resistant CD, the initial response to ustekinumab as observed in 71.4% of patients and maintained up to 6 months (Delgadillo *et al.,* 2016).

Combination therapy

In constant need for improvement and better efficacy of drugs, the performance of drugs to work better in combination was evaluated. Feagan and

co-workers studied the effect of Infliximab and Methotrexate in combination in CD patients who had undergone corticosteroids such as prednisone (Feagan *et al.,* 2014). The findings revealed that the combination therapy was no more effective that Infliximab alone in patients who were undergoing treatment with corticosteroids. Methotrexate was expected to aid the action of Infliximab by inhibiting the formation of antibodies. However, it was assumed that prednisone negated any effect that could have arisen from the usage of Methotrexate and hence the combination was not recommended for patients that require corticosteroids induction. Similar research was carried out by Lémann and co-workers that evaluated the effect of Infliximab in combination with azathioprine (Lémann *et al.,* 2006). In 2013, adalimumab and ciprofloxacin were found to be more effective than adalimumab monotherapy to achieve fistula closure in CD (Dewint *et al.,* 2013). A randomised, double-blind, placebo-controlled trial (ADAFI). However, Osterman and co-workers found an increased risk of non-melanoma skin cancer when adalimumab is co-administered with immunomodulator therapy (Osterman *et al.,* 2014).

Combination therapy with infliximab and azathioprine is significantly more effective for treatment of active Crohn's disease (CD) than infliximab monotherapy. The positive effect of combination therapy was further demonstrated in another randomised clinical study. A SONIC post hoc analysis in 2016 concluded that infliximab and azathioprine given in combination were more effective than drugs given as monotherapy (Colombel *et al.,* 2016). The data further demonstrated that 'deep remission' could be attained with combination therapy in a high percentage of patients with early CD.

Hormone treatments

The anti-inflammatory effects of testosterone have been demonstrated repeatedly in various studies and this effect could account for the patient's improvement by reducing chronic inflammation of the intestinal wall in the CD. A study by Faad et al. (2015) revealed that normalisation of testosterone in hypogonadal men with CD led to improvement from the symptoms associated with the disease. In the same year, Nasser conducted a long-term study on the effect of administration of testosterone undecanoate (a hormone used for the treatment of male hypogonadism) in CD patients while receiving appropriate treatment (Nasser *et al.,* 2015). The results revealed that on receiving testosterone treatment, the Crohn's Disease Activity Index declined from 239.36±36.96 to 71.67±3.26 at 84 months ($p<0.0001$ vs baseline) while not hinge in CDAI was reported in men not receiving the hormonal dose. The data suggested that normalizing serum testosterone levels could help relieve the symptoms of hypogonadal men in CD patients.

The administration of growth hormones for treatment of intestinal disorder was brought to light by researchers such as Byrne and co-workers in 1995 (Byrne *et al.,* 1995) that showed that the intestinal protein absorption for disorders such as short bowel syndrome could be enhanced by the administration of growth hormones like glutamine in combination with amino acid glutamine and proper diet. Slomin and co-workers took this concept a step further and evaluated the efficacy of somatropin; a growth hormone involved in the growth of bones and muscles (Slonim *et al.,* 2000). The study revealed that the CDAI value for hormone-treated group decreased by a mean of 143±144 points while in the placebo group a decrease in only 19±63 points was observed. The benefits of GH treatments were further analyzed in a randomized controlled trial in which 20 pediatric CDtreated with corticosteroids were divided into two groups where one group was treated with 0.075 mg/kg/day Nutropin AQ (somatropin hormone) (Denson *et al.,* 2010). The report found that group treated with GH lead to an improvement in clinical remission as compared to placebo group but no statistical improvement in mucosal inflammation between both groups. The use of hormones offers a safe alternative for the treatment of CD though further studies need to to be carried out for validation.

Antibiotic treatments

The increasing evidence of the role of gut bacteria in CD has shifted the method of treatments from anti-inflammatory drugs to the use of antibiotics. Antibiotics are usually prescribed in mild to moderate CD cases and aim to reduce the symptoms associated with the disease. They work by either reducing the bacterial load in the intestine or by suppressing the immune system of the body. Approved antibiotics which are generally prescribed are rifampicin, ampicillin, sulfonamide, tetracycline while the most common are metronidazole and ciprofloxacin.

Su and colleagues performed a meta-analysis to evaluate the efficacy of antibiotics for the treatment of CD (Su *et al.,* 2015). Of the 15 randomized placebo-controlled clinical trials evaluated, antibiotics benefited CD patients to a certain extent. The findings revealed that ciprofloxacin exhibited significant clinical benefits in patients with perianal fistulas with 95% CI 1.16–2.32. However, showed no statistical significance between CD patients treated with ciprofloxacin and placebo.

Adherent-Invasive E. Coli (AIEC)

A novel pathotype of adherent-invasive

E. coli (AIEC) has been isolated in higher frequency from the ileal mucosa of patients with CD relative to healthy individuals. The ileal mucosa of CD patients is abnormally colonized by AIEC that can adhere to and invade intestinal epithelial cells. In a study, 100 colonies of E.coli were isolated from 20 patients with CD. Mucosa-associated E.coli richness and diversity were found across both controls and CD patients, however, a higher percentage of E.coli was found in CD cases with a higher prevalence of AIEC (CD = 51.9%; C = 16.7%). These data reinforce the idea AIEC plays a crucial role in the CD. Colicin-like bacteriocins are highly potent multidomain protein antibiotics that are often capable of killing susceptible cells at subnanomolar (nM) concentrations.

AIEC forms biofilms in the intestine and can invade host cells and stimulate the production of proinflammatory cytokines. Due to the lack of effective antibiotics for CD treatment, Brown and co-workers (2015) studied the use of species-specific antibiotics (colicins) for treatment of CD (Brown *et al.,* 2015). Colicins displayed strong activity against AIEC bacteria growing as biofilms and also killed cell-associated and intracellular AIEC. The results showed the perspective of colicins as a highly selective probiotic therapy for the eradication of E.coli from the gastrointestinal tract of patients with CD.

***Mycobacterium avium* sub species *paratuberculosis* (MAP)**

The acid-fast gram-positive bacterium is the etiological agent for Johne's disease, an incurable wasting disease prevalent among domestic livestock. The disease is endemic worldwide and causes huge economic loss to the livestock industry. Recently research has highlighted the zoonotic potential of MAP infection and its association with Crohn's disease. Numerous studies have been carried out to understand the link between the two further. Studies have isolated MAP from tissues and have cultured the bacterium from bloodstreams of CD patients. It has been reported that MAP can be cultured from the peripheral mononuclear cells from 50–100% of patients with Crohn's disease and rarely from healthy individuals. The link suggests MAP can either be the cause or an opportunistic pathogen in humans. The polymorphism of Nucleotide binding and Oligomerization Domain 2 (NOD2) is a genetic predisposition involved in CD as well as mycobacterial infections factor (Nabatov., 2015). Nabokov noted that the lack of NOD 2 gene in contributing to CD cases in East Asian population suggestive a secondary involvement in disease development but insist on its significance in the aetiology of CD cases.

However, there are also notable similarities between the symptoms associated with both the diseases in animals and humans. In 2015, Blanche and co-workers aimed to improve the diagnostics of CD through the application of multiple laboratory tests for the detection MAP (Banche *et al.,* 2015). Samples (terminal ileum and colon biopsies, blood and stool) of 19 CD patients and 11 healthy individuals (controls) were subjected to staining, culture and IS900 PCR and the frequency of MAP was significantly higher in CD than in CD patients and thus suggested MAP has the possibility to be used as a marker for CD.

Many studies over the years have linked the imbalance in gut microbes with CD. A study in 2004revealed that persons suffering from IBD were more likely to receive antibiotic treatments before IBD diagnosis than healthy individuals suggesting a connection between the two categories rather than a cause (Card *et al.,* 2004). The association of gut microbes and CD was further revealed in a study by Bernstein (Bernstein, 2013). An individual's gut flora develops during the 1st year as a child and then remains relatively the same. Any infection during the 1st year could affect the gut microbes and cause permanent change. A review was conducted based on all persons in Manitoba, Canada with a documented diagnosis of CD and ulcerative colitis from 1984 to 2008. Of the 36 subjects, children who were diagnosed with IBD were three times more likely than those without IBD to have had taken antibiotic treatments during the 1st year of life. Using shotgun metagenomic sequencing Lewis and colleagues analyzed faecal samples of potential pediatric CD patients and studied the entire microbial community during treatment. The study revealed similar results wherein antibiotic exposure was directly associated with increased dysbiosis (abnormal composition of intestinal bacteria) (Lewis *et al.,* 2015). Fungal proportion also increased with the use of antibiotics and on the severity of the disease. A study by Xavier et al. (2016) showed an imbalance in microbial flora in the intestine of patients suffering from CD wherein some microbes were found in lesser amount while other harmful microbes flourished. Ungaro and colleague performed a meta-analysis studying the antibiotic exposure as a risk factor for developing IBD (Unagaro *et al.,* 2015). 11 observational studies (8 case-control and 3 cohorts) and 7,208 patients diagnosed with IBD were analyzed and statistical analyses revealed that antibiotic exposure was significantly associated with CD though not significantly associated with UC. All antibiotics in the

study (Metronidazole, fluoroquinolones, broad-spectrum, penicillins, tetracyclines, cephalosporins, macrolides, sulphonamides, and penicillin) had a certain level of association with the new onset CD through the highest level was found in metronidazole and fluoroquinolones. The study is quite startling because antibiotics like metronidazole are used for the treatment of CD and change the focus in which treatments are considered.

TGF therapy

Crohn's disease can also be attributed to the defects in the transforming growth factor (TGF)-β1 signalling pathways that are cytokine immunosuppressive secreted proteins that are involved in many cellular functions in the body. TGF β1- is produced in the human gastrointestinal tract of healthy individuals in a large amount and plays a decisive role in negatively controlling inflammatory pathways. Research has shown that phosphorylated smad3 is highly expressed in LPMC (Lamina propria mononuclear cells) isolated from healthy colon and responded to exogenous TGF-B1 with the inhibition of inflammatory cytokines. However, the upregulation of smad7 blocks phosphorylation of Smad 3 and invariantly inhibits TGF-B1 signalling. Hence, it is assumed that blocking smad7 will result in the TGF-β1-induced Smad2/3 activation and negatively control inflammation. The notion was confirmed in a study where Knockdown of Smad7 with a specific antisense oligonucleotide restoresdTGF-β1/Smad3 signalling, thus resulting in a marked suppression of inflammatory cytokine production (Izzo *et al.,* 2018). In 2006 Boirivant and coworkers studied the effect of oral smad7 on mucosal inflammation in the colitic tissues of mice. The data suggested that defective TGF β1 signalling was due to the high levels if smad7 and on downregulation of the secretory protein the TGF β1 levels were restored (Boirivant *et al.,* 2006).

Recently, a formulation of Mongers on (ED)is under clinical trials for the cure of CD with promising results. Mongerson formulation consists of a 21-base single-strand phosphorothioate oligonucleotide that will hybridize to the human *SMAD7* messenger RNA (mRNA) which then facilitates RNase H–mediated RNA degradation through a standard antisense mechanism (Ardizzone *et al.,* 2016). In the phase 1 study of 15 patients with CD, a decrease of more 70 points in the CD Activity Index score was observed in patients who were administered with the drug. In phase II, clinical trial the efficacy of the drug was evaluated and the study revealed that the participants with CD who received mongererson had higher remission rate than control groups (Monteleone *et al.,* 2015). Currently, the researchers are evaluating the colonoscopic results of the patients who were administered with the drug and Celgene, the company sponsoring the project is under process for hiring 2,000 CD patients for the final phase III trials. As a pill, the drug has a potential to become the preferred therapy over other available injected drugs (Humira or Remicade)

Hematopoietic Stem Cell Transplantation (HSCT)

During the 1990's, reports have shown that patients suffering from IBD have a statistically higher chance of developing. As a result, it was observed that a higher number of IBD patients would require to undergo bone marrow transplantation. In 1993, Darko's and co-workers reported of a 41-year-old female patient who had Crohn's disease, had to under autologous BMT as she was also suffering from non-Hodgkin's lymphoma (NHL) (Darkos *et al.,* 1993) intermediate grade. It was observed that 18 days post-BMT; the patient attained complete remission for CD, was healthy, and in 6-month follow up period required no treatment for the CD. Over the years, several animal models have revealed that Hematopoietic Stem Cell transplantation (HSCT) is very useful for the treatment of autoimmune disorders. In this regard, many cases have been reported of successful remission induced in patients with (therapy refractory) Crohn's disease using an autologous HSCT.

A recent case report highlights the significance of HSCT treatment. Ruiz and co-workers in 2015 reported a case of a 28-year-old woman with refractory CD (Ruiz *et al.,* 2015). The patient was subjected to autologous hematopoietic stem cell transplantation (HSCT) and all the symptoms associated with the disease such as diarrhoea and abdominal and joint pain disappeared during the conditioning regimen or immediately after HSCT procedure. The patient was mobilized with cyclophosphamide (60 mg/kg) and granulocyte colony stimulating factor (10 mg/kg) for seven days with 4.59 × 106 CD34+ cells/kg being collected from peripheral blood in two leukapheresis sessions (Hemonetics MSC+USA). The patient attained clinical remission until the end of the report i.e. one year after the treatment.

Oyama and co-workers performed extensive research on HSCT for the treatment of CD based on the concept that maximum immune ablation by autologous hematopoietic stem cell transplantation (HSCT) could induce remission in patients (Oyama *et al.,* 2005). In the phase 1 study, 12 patients with refractory CD were chosen, who were initially treated with infliximab and failed to respond to the treatment. In this study, peripheral blood stem cells were mobilized with cyclophosphamide

(medication used to decrease the immune system response) and granulocyte colony-stimulating factor and CD34 + enriched. 11 of the 12 patients attained remission and after a follow up of 18.5 months, only one patient had a recurrence of the CD. In 2010 the group went further to study the efficacy of high-dose cyclophosphamide, antithymocyte globulin (ATG), and autologous $CD34^+$ cell–enriched HSC in chronic active CD patients that did not get a response to anti-TNF therapy (Burt *et al.,* 2010). The results revealed that the patients achieved treatment-free remissions for as long as 5 years. Hommes and co-workers studied the long-term effect of autologous hematopoietic stem cell transplantation for the severe refractory CD (Hommes *et al.,* 2011). All the 3 patients in the study achieved remission under treatment and concluded that autologous HSCT appeared to be a safe and alternative strategy for CD treatments but cautioned that due to the high mortality rate of HSCT patients, the treatment must only be considered when all other options fail. The long-term effect was also evaluated by Snowden and co-workers (Snowden *et al.,* 2014). Following stem cell mobilization, patients were treated with high-dose cyclophosphamide (200 mg/kg) and rabbit anti-thymocyte globulin (7.5 mg/kg) followed by ASCT. Clinical remission was achieved at 3 months for five or six patients and the authors concluded that ASCT was a feasible and safe treatment for the long-term control of severe CD and additional it was noted that the tradesmen resensitized the disease to medical therapy lowering the probability of surgery.

The feasibility and toxicity of autologous HSCT were evaluated by Jauregui-Amezaga and co-workers (Jauregui-Amezaga *et al.,* 2015). 26 patients were involved in the study and the toxicity and complication that arose due to the treatment were evaluated. During mobilization and conditioning patients suffered from infectious complication ranging from neutropenia, bacteraemia, septic shocks and non-infectious complication like antithymocyte globulin reaction, mucositis and haemorrhagic complications. Viral infection was the predominant concern during the first twelve months of treatment and one patient had died due to systemic cytomegalovirus infection. Highlighting the disadvantages associated with HSCT, Hawkey and co-workers evaluated the effect of autologous HSCT on refractory Crohn disease and compared using standard CD treatment (Hawkey *et al.,* 2015). The authors concluded that there were 76 adverse events occurred in patients undergoing HSCT while 38 such events occurred in control. All these findings point to the fact that though Autologous HSCT for patients with refractory CD is feasible, the reports do not support the widespread use of HSCT for patients with refractory CD and due to a large number of complication that could arise the proce- dures should only be performed in highly experi- enced centres.

A promising therapeutic approach is(C-Chemokine receptor type 9) CCR9 antagonism. Expressed on the cell surface of memory/effector CD4(+) T cells these selectively binds to the small intestinal lymphocyte chemoattractant CCL25. Blockade of the CCR9/CCL25 interaction inhibits lymphocyte homing to the intestinal mucosa, limiting the inflammation and disease at this site. Vercirnon, a selective CCR9 antagonist drug is currently under development. In 2013, Arseneau and co-workers gave an expert opinion suggesting that if the results of ongoing large-scale clinical trials of vercirnon are in line with preliminary reports, CCR9 antagonism may have comparable efficacy to anti-TNF therapies and a potentially superior safety profile, making it the latest addition to the growing arsenal of immunomodulatory drug therapies available to combat Crohn's disease. Moreover, its economic benefit since vercirnon is an oral drug, and its associated costs will likely be much lower than expensive infusion-based anti-TNF therapies, providing further economic benefits. Feagan and co-workers in 2015 were not able to demonstrate the efficacy of vercirnon as induction therapy in patients with moderately-to-severely active CD (Feagan *et al.,* 2015).

Complement and Alternative Medicine (CAM)

Most of the available maintenance therapy focus on lowering the immune system, a potential hazard to patients as this could make people susceptible to other infections. As the disease lacks an absolute cure and the patients usually have to deal with the conditions throughout their life, the use alternative medicines; natural products derived from plants and animals are slowly gaining popularity among CD patients. A brief outline is mentioned below.

Fish Oil

Omega-3 polyunsaturated fatty acids (n-3) mainly found in fish oil has been suggested as an alternative therapy for the CD. Though conflicting results in different trails have been noted, more studies need to be carried out to confirm either case. The anti-inflammatory properties associated with fish oil has lead to the suggestion in the early 1900's as an alternative treatment for the CD.

Earlier studies such as Belluzzi and co-workers in 1996 highlights the therapeutic benefits of administration of n-3 to CD patients. 59 % of the fish oil group were in remission after one-year in comparison to 26% in placebo. A novel capsule was used in the study that prevented the breakdown by gastric acidity for 30 minutes so that the fish oil can be

released into the small intestine. Other studies supported these results as well that showed the beneficial aspect of n-3 for CD patients (Tsujikawa *et al.,* 2000). However, unlike in animal models, which were more consistent, many human trials have shown conflicting results. For, e.g., in 1996 clinical trial conducted by Lorenz and co-workers concluded that n-3 did not prolong the remission in CD patients. Epanova Program in Crohn's Study 1[EPIC trials], a multicentre study conducted between 2003 and 2007, is highly regarded as an adequate and well-designed study, failed to establish any positive effect that arouses from the administration of n-3. Nonetheless, as suggested by Tsujikawa in 2000, the low percentage of CD patients in Japan it could be due to the diet rich in fish and the increase in CD patients de to shift in diet hence further work still needs to be done as the trials differ in different aspects, from concentration of n-3 to the type of capsules used.

Herb Based treatments

Tripterygium wilfordii vine, traditionally used in Chinese medicine for treatment of wide range of illness have indicated for its therapeutic characteristics against CD. Ren and co-workers evaluated the efficacy of T2, the main component of the extracts of the plants in 2007 for the treatment of CD (Ren *et al.,* 2007). CDAI scores rapidly improved with the decrease in the serum levels of CRP, TNFa, and IL-1beta. The same group in 2013 evaluated the effectiveness polyglycosides of *Tripterygium wilfordii* (GTW) and compared it with mesalazine (5-ASA) in preventing postoperative clinical and endoscopic recurrence of Crohn's disease (Ren *et al.,* 2013)). The findings supported their claims and additionally stated that the vine extract seemed to be superior to 5-ASA treatment regimen. In 2015 the herb was further compared against azathioprine and report suggests that the TWHF was comparable to azathioprine for preventing postoperative clinical recurrence Zhu *et al.,* 2014.

Cannabis is a herb commonly known for its recreational use and is known to be beneficial to patients with IBD. The first controlled trial was performed by Naftali and co-workers, where patients were given cannabis in the form of cigarettes containing 115 mg of Δ9-tetrahydrocannabinol (THC) two times on a daily basis (Naftali *et al.,* 2013). 5 of the 11 patients achieved complete remission (CDAI score, <150) in comparison to 1 in 10 of the placebo group. Though more needs to be done, the study suggests an alternative mechanism for the treatment of CD. It has been reported that cannabis use.

Native to India and Pakistan *Boswellia serrata* is commonly used in the production of Indian frankincense. Studies have revealed that the pure compound isolated from the plant have anti-inflammatory properties by down-regulating TNFα, IL-1β, and NO (Gayathri *et al.,* 2007). In 2001, the efficacy of *Boswellia serrata* extract H15 was compared with mesalazine for the treatment of active CD. The authors concluded that taking into consideration benefit-risk evaluation, the extract was superior to mesalazine treatment but lacks in statistical significance in a change in CDAI score after administration of the drugs. (drop in 90 points for extract group and 53 points in mesalazine group) (Gastroenterol *et al.,* 2013). The most recent work was performed by Holtmeier, who tried to evaluate the efficacy of the extract in maintaining remission (Holtmeier *et al.,* 2011). Like the previous work, the extract was well tolerated among CD patients but failed to prove any advantage in comparison with the placebo group.

Pistacia lentiscus var. Chia (Anacardiaceae) also known as Chios Mastic Mastiha, is an evergreen shrub widely used in culinary purposes. Its antioxidant, anticancer and antibacterial properties, the extract is widely known to treat a wide range of illness. In 2007, Kaliora and co-workers conducted a pilot study where the effectiveness of mastic extract was evaluated for the treatment of CD (Kalioraet *al.,* 2007). Ten CD patients were treated with mastic caps (6 caps/d, 0.37 g/cap) for 4 weeks and patients exhibited a significant reduction of CDAI (222.9 ± 18.7 *vs* 136.3 ± 12.3) values compared to pretreatment values. No recent work has been carried out for evaluating the efficacy of the extract and by conducting more extensive studies.

Artemisia *absinthium* also has known as common wormwood has been reported to suppress tumour necrosis factor alpha (TNF-α) and other interleukins in in-vitro studies. A clinical trial in 2007 noted complete remission in 65% of the patients after 8 weeks of treatment with a herbal blend containing wormwood herb (3×500 mg/day) along with steroids. The amount of steroids was gradually reduced and by week 10, no steroids were administered. The study also highlighted that the herb has a steroid-sparing effect. Further evaluation was conducted in 2010 where 10 CD patients were administered with 3×750 mg dried powdered wormwood for 6 weeks along with the standard medications along with 10 patients who did not receive the herb was taken as control. The serum TNF a level was 8.0±2.5 pg/ml after six weeks for the herbal group in comparison to 21.1±3.2 (week 6) for the control group. THE CDAI scores were declined from 275±15 to below 175±12 in the herbal group with remission attained in 8 patients compared to only two in the

placebo group, which attained remission. However, promising, more extensive studies must be carried out to confirm its efficacy.

Conclusion:

This Study Givens, the High global incidence of CD disease, has been reported in India and its advances in modern-day research.

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